# A New *SPINK5* Donor Splice Site Mutation in Siblings with Netherton Syndrome

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*Sir,*

Netherton syndrome (NS, OMIM 256500) is a rare autosomal recessive genodermatoses, characterized mainly by ichthyosiform erythroderma, a specific hair shaft defect (i.e. trichorrhexis invaginata) and atopic manifestations (1). The skin involvement presents at birth or shortly afterwards in the form of a generalized exfoliative erythroderma, which can either persist throughout life or gradually evolve in childhood into a milder condition, known as ichthyosis linearis circumflexa (ILC). ILC consists of migratory, serpiginous and erythematous plaques surrounded by a peculiar double-edged scale (1). Bacterial infections of the skin, upper and lower airways, hypernatraemic dehydration, electrolyte imbalances, hypothermia and failure to thrive frequently occur in NS and account for a relatively high postnatal mortality (2).

NS is caused by loss-of-function mutations in the *SPINK5* gene encoding LEKTI, a multi-domain serine protease inhibitor whose activity is crucial for the formation and physiological renewal of the epidermal barrier (3–5). In the skin, LEKTI is normally detected in the most differentiated layers of the epidermis, and loss of LEKTI expression has been shown to be diagnostic for NS (4, 6). Screening of the *SPINK5* gene in NS families has led to the identification of a number of distinct mutations, some of which are common either within specific populations or among different ethnic groups. We report here two male siblings affected by NS, which resulted from a previously undescribed splicing mutation in *SPINK5*.

## CASE REPORT AND RESULTS

Patient 1 was a 6.5-year-old child, while patient 2 was one year younger. The parents were consanguineous (first cousins) and of Turkish origin. Both patients were born at term after an uneventful pregnancy and delivery. Birth measurements were all within normal limits. The patients were referred to us for a long history of a waxing and waning, but never clearing, generalized skin eruption and recurrent pulmonary infections, which both started shortly after birth. On physical examination, the scalp of both patients appeared diffusely scaling with short, brittle hair and sparse eyebrows (Fig. 1a). Erythematous desquamating plaques were evident on the face, arms, hand dorsum, inguinal and intergluteal folds. In addition, the trunk and legs presented numerous polycyclic erythematous patches with double-edged scales (Fig. 1b). Teeth, nails eyes and mucous membranes were normal. Laboratory tests demonstrated extremely high levels of serum IgE in both patients (11,300 and 12,500 IU/ml, respectively). Light microscopy observation of the scalp hair of both patients showed the typical trichorrhexis invaginata.

A skin biopsy from both siblings and blood samples from the nuclear family were obtained after written informed consent. Genomic DNA extracted from lymphocytes was used to amplify and sequence all the 33 *SPINK5* exons and flanking intronic borders, as described previously (6).

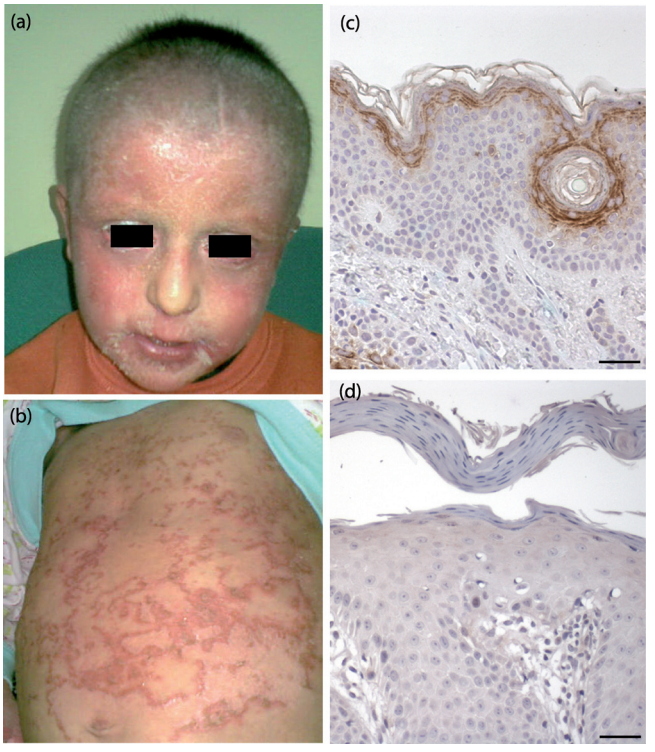


*Fig. 1.* The younger sibling with Netherton syndrome. (a) Diffuse scaling of the scalp with short, lustreless, brittle hair; erythematous desquamating lesions of the face with angular cheilitis, blepharitis and sparse eyebrows and eyelashes. (b) Lesions of ichthyosis linearis circumflexa of the trunk. (c, d) Immunohistochemical detection of LEKTI in the skin of the older sibling (d). Normal control (c) with the 1C11G6 antibody. Strong LEKTI expression is evident in the granular layer of the control epidermis and absent in the patient. Note the cleavage within the lower layers of the parakeratotic stratum corneum. Scale bar = 40 μm. The patient has approved publication of this figure.

Histological examination of the skin revealed psoriasiform epidermal hyperplasia with parakeratosis, mild focal spongiosis, and a dermal inflammatory infiltrate. LEKTI expression was evaluated by immunohistochemistry using a commercial monoclonal antibody generated against the entire recombinant protein (clone 1C11G6, Zymed Laboratories. San Francisco, USA) and a polyclonal antibody recognizing a C-terminal fragment (anti-LEKTI D13-15) (4, 6), as previously described. No immunoreactivity was detected with either of the two antibodies, thus providing further evidence for the diagnosis of NS (Fig. 1c and d). Mutation search in the *SPINK5* gene revealed the presence of a novel 4-bp deletion affecting the donor splice site of intron 25 (c.2441+3delGAGT; GenBank no. AJ228139.2). The mutation was present in homozygosity in both affected siblings and in heterozygosity in their healthy parents, and was not detected in 51 ethnically matched healthy subjects (Fig. 2a).

The consequences of the c.2441+3delGAGT mutation on pre-mRNA splicing were evaluated by computational prediction

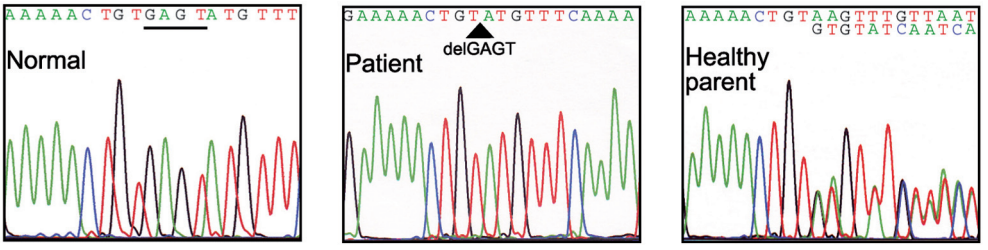


*Fig. 2.* Molecular findings in the family with Netherton syndrome (NS). Direct sequencing of polymerase chain reaction (PCR) products spanning the SPINK5 exon 25 and its flanking splice site junctions shows the presence of a 4-bp deletion (c.2441+3delGAGT) within the donor splice site of intron 25, at the homozygous state in the affected children (*central panel*) and at the heterozygous state in the healthy parents (*right-hand panel*). The GAGT deletion is not detected in the equivalent region of a control DNA (*left-hand panel, underlined sequence*).

using two different software programs available at http://www.fruitfly.org/ and http://linux1.softberry.com). Both predictors showed that the splice site score of the mutant sequence was much lower (0.59) than the wild-type counterpart (0.96). This probably hampers its recognition by the splicing machinery, thus prefiguring either the utilization of downstream intronic cryptic donor splice sites (positions +31 and +102 in the wild-type sequence), which are stronger than the mutant one (score of 0.67 and 0.88, respectively), or the skipping of the entire exon 25 (electronic Fig. 3a; http://adv.medicaljournals.se/article/abstract/10.2340.00015555-0769/fig3). Each of these outcomes leads to premature stop codon formation, resulting in LEKTI truncated polypeptides and/or marked instability of the mutant transcripts.

## DISCUSSION

To date, several consanguineous unrelated NS Turkish patients have been described. They were all homozygous for the recurrent *SPINK5* c.153delT deletion, which results in LEKTI truncation within the first serine protease inhibitory domain (7, 8). In the majority of these patients the NS phenotype was severe and often lethal during the neonatal period because of dehydration and sepsis. Although no clear genotype-phenotype correlation has been defined in NS, it is conceivable that the less severe phenotype seen in our patients may partly depend on the mutation site, which allows for retention of LEKTI domains 1–11. Although truncated LEKTI immunoreactivity in NS tissue is difficult to detect with currently available antibodies, a correlation between *SPINK5* mutation position and specific clinical features has been described in Japanese as well as other patients (9, 10).

In conclusion, the c.2441+3delGAGT splice site mutation in the present family contributes to delineate a *SPINK5* mutational spectrum in the Turkish population, and adds to the global mutation database, which at present comprises 60 different mutations (electronic Fig. 3b; http://adv.medicaljournals.se/article/abstract/10.2340.00015555-0769/fig3).

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